CLAIMS

What is claimed is:

1. A method of determining the presence and sequence of at least one target polynucleotide in a sample comprising:

combining nucleic acid from the sample with at least one reaction composition comprising a fluorescent indicator and amplification primers specific to the at least one target polynucleotide;

amplifying the at least one target polynucleotide present in the reaction composition using the amplification primers to obtain at least one amplification product;

irradiating the at least one amplification product such that the fluorescent indicator produces a fluorescent signal, wherein the intensity of the fluorescent signal is related to the quantity of the at least one amplification product;

monitoring the amplifying by detecting the fluorescent signal from the fluorescent indicator;

determining whether the at least one amplification product is present from the intensity of signal from the fluorescent indicator; and

determining the sequence of the at least one amplification product if the at least one amplification product is present.

2. The method of claim 1, wherein the determining of the sequence comprises:

performing a sequencing reaction on the at least one amplification product to obtain a sequencing product; and

placing the product of the sequencing reaction into a sequencing apparatus to determine a sequence of the at least one amplification product.

3. The method of claim 2, wherein the fluorescent signal is detected using a device.

4. The method of claim 3, wherein the amplifying, irradiating, and monitoring comprise use of a thermal cycler, a device that irradiates the at least one amplification product, a device that detects resulting fluorescence during each cycle, and a device that displays the increase in fluorescence by cycle number.

5. The method of claim 4, wherein the thermal cycler, the device that irradiates, the device that detects, and the device that displays are all components of a single device.

6. The method of claim 4, wherein the amount of amplification product and number of cycles are used to determine the amount of target polynucleotide present in the sample prior to the amplification.

7. The method of claim 3, wherein nucleic acid from the sample is combined with at least two different separate reaction compositions, wherein each reaction composition comprises a fluorescent indicator and a different set of amplification primers specific to a different target polynucleotide, and wherein the amplifying results in different amplification products if the different target polynucleotides are present in the sample.

8. The method of claim 3, wherein the monitoring occurs after the amplifying is complete.

9. The method of claim 4, wherein the monitoring occurs during two or more cycles during the amplifying.

10. The method of claim 3, wherein the nucleic acid from the sample is derived from a group comprising a virus, a prokaryote, a protist, a plant, a fungus, and an animal.

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11. The method of claim 3, wherein the presence of a given target polynucleotide indicates the presence of a pathogen selected from at least one of a virus, a prokaryote, and a eukaryote.

12. The method of claim 11, wherein the pathogen is selected from at least one of HIV, specific *E. coli* strains, *Salmonella sp.*, and *Haemophilus sp.*

13. The method of claim 3, wherein the presence of a given target polynucleotide indicates the presence of at least one of a genetic disease or a genetic predisposition to a disease.

14. The method of claim 3, wherein the presence of a given target polynucleotide indicates the presence of a specific allele.

15. The method of claim 14, wherein the presence of the specific allele indicates serotype.

16. The method of claim 14, wherein the presence of one or more specific alleles indicate one or more cell surface proteins which determine at least one HLA type.

17. The method of claim 16, wherein the at least one HLA type comprises at least one of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-DRA, HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1, HLA-DOA, HLA-DOB, HLA-DMA, and HLA-DMB.

18. The method of claim 3, wherein the presence of a given target polynucleotide indicates the presence of at least one of TAP1, TAP2, and MICA.

19. The method of claim 3, wherein the fluorescent indicator is a nucleic acid binding molecule.

20. The method of claim 19, wherein the fluorescent indicator is an intercalating dye.

21. The method of claim 19, wherein the fluorescent indicator is a minor groove binding dye.

22. The method of claim 19, wherein the fluorescent indicator is a molecular beacon.

23. The method of claim 3, wherein the fluorescent indicator is selected from a group comprising SYBR® Green I; thiazole orange; ethidium bromide; pico green; acridine orange; quinolinium 4-[(3-methyl-2(3H)-benzoxazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; quinolinium 4-[(3-methyl-2(3H)-benzothiazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; and chromomycin A3.

24. The method of claim 3, wherein the fluorescent indicator is a 5'-nuclease fluorescent indicator.

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25. The method of claim 3, wherein the sample is selected from a group comprising whole blood, a tissue biopsy, bone marrow, semen, sputum, urine, amniotic fluid, sperm, hair, skin, and cultured cells.

26. A method of determining the presence and sequence of at least one target polynucleotide in a sample comprising:

combining nucleic acid from the sample with at least one set of reaction compositions comprising a first reaction composition and second reaction composition, both specific for the at least one target polynucleotide, wherein the first reaction composition comprises amplification primers specific to the at least one target polynucleotide, and the second reaction composition comprises a fluorescent indicator and amplification primers specific to the at least one target polynucleotide;

amplifying the at least one target polynucleotide present in the reaction compositions using the amplification primers to obtain at least one amplification product;

irradiating the at least one amplification product of the second reaction composition such that the fluorescent indicator produces a fluorescent signal, wherein the intensity of the fluorescent signal is related to the quantity of the at least one amplification product;

monitoring the amplifying of the second reaction composition by detecting the fluorescent signal from the fluorescent indicator;

determining whether the at least one amplification product is present in both the first reaction composition and the second reaction composition from the intensity of signal from the fluorescent indicator in the second reaction composition; and

determining the sequence of the at least one amplification product of the first reaction composition if the at least one amplification product is present in the first reaction composition.

27. The method of claim 26, wherein the determining of the sequence comprises:

performing a sequencing reaction on the at least one amplification product to obtain a sequencing product; and

placing the product of the sequencing reaction into a sequencing apparatus to determine a sequence of the at least one amplification product.

28. The method of claim 27, wherein the fluorescent signal is detected using a device.

29. The method of claim 28, wherein the amplifying, irradiating, and monitoring comprise use of a thermal cycler, a device that irradiates the at least one amplification product, a device that detects resulting fluorescence during each cycle, and a device that displays the increase in fluorescence by cycle number.

30. The method of claim 29, wherein the thermal cycler, the device that irradiates, the device that detects, and the device that displays are all components of a single device.

31. The method of claim 28, wherein nucleic acid from the sample is combined with at least two different sets of separate reaction compositions comprising the first reaction composition and the second reaction composition, wherein each set of separate reaction compositions comprises amplification primers specific for a different target polynucleotide, and wherein the amplifying results in different amplification products if the different target polynucleotides are present in the sample.

32. The method of claim 29, wherein the amount of amplification product and number of cycles are used to determine the amount of target polynucleotide present in the sample prior to the amplification.

33. The method of claim 28, wherein the monitoring occurs after the amplifying is complete.

34. The method of claim 29, wherein the monitoring occurs during two or more cycles during the amplifying.

35. The method of claim 28, wherein the nucleic acid from the sample is derived from a group comprising a virus, a prokaryote, a protist, a plant, a fungus, and an animal.

36. The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of a pathogen selected from at least one of a virus, a prokaryote, and a eukaryote.

37. The method of claim 36, wherein the pathogen is selected from at least one of HIV, specific *E. coli* strains, *Salmonella sp.*, and *Haemophilus sp.*

38. The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of at least one of a genetic disease or a genetic predisposition to a disease.

39. The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of a specific allele.

40. The method of claim 39, wherein the presence of the specific allele indicates serotype.

41. The method of claim 39, wherein the presence of one or more specific alleles indicate one or more cell surface proteins which determine at least one HLA type.

42. The method of claim 41, wherein the at least one HLA type comprises at least one of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-DRA, HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1, HLA-DOA, HLA-DOB, HLA-DMA, and HLA-DMB.

43. The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of at least one of TAP1, TAP2, and MICA.

44. The method of claim 28, wherein the fluorescent indicator is a nucleic acid binding molecule.

45. The method of claim 44, wherein the fluorescent indicator is an intercalating dye.

46. The method of claim 44, wherein the fluorescent indicator is a minor groove binding molecule.

47. The method of claim 44, wherein the fluorescent indicator is a molecular beacon.

48. The method of claim 28, wherein the fluorescent indicator is selected from a group comprising SYBR® Green I; thiazole orange; ethidium bromide; pico green; acridine orange; quinolinium 4-[(3-methyl-2(3H)-benzoxazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; quinolinium 4-[(3-methyl-2(3H)-benzothiazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; and chromomycin A3.

49. The method of claim 28, wherein the fluorescent indicator is a 5'-nuclease fluorescent indicator.

50. The method of claim 28, wherein the sample is selected from a group comprising whole blood, a tissue biopsy, bone marrow, semen, sputum, urine, amniotic fluid, sperm, hair, skin, and cultured cells.

51. A kit for performing amplification and sequencing reactions on a sample, comprising:

amplification primers designed for amplification of at least one target polynucleotide in a sample to obtain at least one amplification product;

a fluorescent indicator whose intensity is related to the amount of amplification product generated in an amplification reaction; and

sequencing primers specific to the at least one amplification product.

52. The kit of claim 51, wherein the fluorescent indicator is a nucleic acid binding molecule.

53. The kit of claim 52, wherein the fluorescent indicator is an intercalating dye.

54. The kit of claim 52, wherein the fluorescent indicator is a minor groove binding molecule.

55. The kit of claim 52, wherein the fluorescent indicator is a molecular beacon.

56. The kit of claim 51, wherein the fluorescent indicator is selected from a group comprising SYBR® Green I; thiazole orange; ethidium bromide; pico green; acridine orange; quinolinium 4-[(3-methyl-2(3H)-benzoxazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; quinolinium 4-[(3-methyl-2(3H)-benzothiazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; and chromomycin A3.

57. The kit of claim 51, wherein the fluorescent indicator is a 5'-nuclease fluorescent indicator.

58. The kit of claim 51, wherein the kit further comprises sequence terminators for performing a sequencing reaction.

59. The kit of claim 58, wherein the sequence terminators are fluorescently labeled.

60. The kit of claim 51, wherein the sequencing primers are labeled.

61. The kit of claim 51, wherein the amplification products are used to determine the presence of a specific allele.

62. The kit of claim 61, wherein the presence of the allele indicates serotype.

63. The kit of claim 62, wherein the presence of one or more specific alleles indicate one or more cell surface proteins which determine at least one HLA type.

64. The kit of claim 63, wherein the at least one HLA type comprises at least one of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-DRA, HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1, HLA-DOA, HLA-DOB, HLA-DMA, and HLA-DMB.

65. The kit of claim 51, wherein the presence of a given target polynucleotide indicates the presence of at least one of TAP1, TAP2, and MICA.

66. The kit of claim 51, wherein the amplification product indicates the presence of a pathogen in the sample.

67. The kit of claim 66, wherein the pathogen is selected from a group comprising a virus, a prokaryote, and a eukaryote.

68. A method of determining the presence and sequence of at least one target polynucleotide in a sample comprising:

combining nucleic acid from the sample with at least one reaction composition comprising an intercalating fluorescent indicator and amplification primers specific to the at least one target polynucleotide;

amplifying by polymerase chain reaction the at least one target polynucleotide present in the reaction composition using the amplification primers to obtain at least one amplification product;

irradiating the at least one amplification product such that the intercalating fluorescent indicator produces a fluorescent signal, wherein the intensity of the fluorescent signal is related to the quantity of the at least one amplification product;

monitoring the amplifying by using an apparatus that detects the fluorescent signal from the intercalating fluorescent indicator;

determining whether the at least one amplification product is present from the intensity of signal from the fluorescent indicator;

performing a sequencing reaction on the at least one amplification product if the at least one amplification product is present to obtain a sequencing product; and

placing the product of the sequencing reaction into a sequencing apparatus to determine a sequence of the at least one amplification product.